



Histamine plasma levels and elimination diet in chronic idiopathic urticaria

B Guida^{1*}, C De Martino¹, S De Martino², G Tritto², V Patella³, R Trio¹, C D'Agostino¹, P Pecoraro¹ and L D'Agostino²

¹Department of Physiology, Nutrition Section, University 'Federico II', Naples, Italy; ²Department of Gastroenterology, University 'Federico II', Naples, Italy; ³Department of Immunology, University 'Federico II', Naples, Italy

Objective: The aim of this study was to evaluate the effects of an oligoantigenic and histamine-free diet on patients affected with chronic idiopathic urticaria (CIU).

Design: Ten patients with chronic idiopathic urticaria were prescribed an oligoantigenic and histamine-free diet for 21 days, followed by serial and controlled reintroduction of foods during a further 70 days. Modification in clinical illness as well as histamine plasma levels, post-heparin plasma diamine oxidase (DAO) and intestinal permeability were evaluated.

Results: The oligoantigenic and histamine-free diet induced a significant improvement of symptoms ($P < 0.05$). Moreover, CIU patients on free diet showed higher histamine plasma levels ($P < 0.05$ vs post-diet and vs controls) that fell to control levels during the oligoantigenic and histamine-free diet. Post-heparin plasma diamine oxidase values were slightly reduced and were unchanged during the diet as well as intestinal permeability, which was always normal in all patients.

Conclusions: These data suggest that histamine plays a major role in chronic idiopathic urticaria. The finding of normal intestinal permeability suggests that a morphological damage of intestinal mucosa should be excluded in these patients. However, the presence of low levels of post-heparin plasma diamine oxidase may indicate a subclinical impairment of small bowel enterocyte function that could induce a higher sensitivity to histamine-rich or histamine-producing food.

Descriptors: chronic idiopathic urticaria; diet; histamine; diamine oxidase; intestinal permeability
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Introduction

Urticarias are a complex group of disorders characterised by itchy wheals or swelling of the skin. In 70–95% of patients the cause of chronic urticaria remains undetermined. Chronic idiopathic urticaria (CIU) is defined as repeated episodes or even continuous urticaria of unknown origin (Champion, 1988; Kaplan, 1988) and without any evidence of IgE mediated reaction (Warin, 1976). About 30–40% of patients with chronic urticaria attribute their symptoms to food intolerance (Julin, 1981; Sayag *et al*, 1988), even if the affected person is seldom able to exactly identify the causative food. The possible pathogenic role of pseudoallergic reactions to food has been repeatedly discussed. The term 'pseudo food allergy' is usually used for reactions involving histamine or mast cell degranulation as a mechanism in which the primary stimulus is non-immunologic (i.e. not IgE mediated; Smith *et al*, 1995; Barlow *et al*, 1995; Bischoff *et al*, 1992; Claveau *et al*, 1993; Zweiman *et al*, 1998).

Mast cells are considered the primary effector cells in urticaria. The typical symptoms can be attributed to the effect of histamine and other mast cell mediators on local

tissue constituents. It has been suggested that pseudoallergic manifestations are not only the result of mast cell activation, but also a consequence of a defect in histamine metabolism (Lessoft, 1991). Histamine is metabolized by two pathways, oxidation and methylation. N-methyl transferase methylates histamine in the skin and is active in the liver. Diamine oxidase (DAO) normally degrades histamine, methyl histamine and diamine and it is confined to the small bowel mucosa. The loss of the protective role of mucosal DAO might allow an increased absorption of biological amines.

In the past few years, several reports on the usefulness of the plasma post-heparin diamine oxidase (PHD) test in evaluating the small intestinal mucosa integrity have been published (D'Agostino *et al*, 1987a, b; Corazza *et al*, 1988; Thompson *et al*, 1988). The test is based on the ability of heparin to release the enzyme diamine oxidase (DAO) from the small intestinal mucosa into the circulation (Shakir *et al*, 1977; D'Agostino *et al*, 1989). Since the enzyme is synthesized by the differentiated and non-proliferating enterocytes, the enzyme activity in the blood after an intravenous injection of heparin correlates with the morphologic integrity of the small intestinal epithelium (D'Agostino *et al*, 1987a, b; Luk *et al*, 1983). The test proved to be helpful in quantitating the remaining mature enterocyte mass in patients with celiac disease, before (D'Agostino *et al*, 1987b; Corazza *et al*, 1988) and after (D'Agostino *et al*, 1987b) gluten-free diet, small bowel lymphoma (Salmeron & Modigliani, 1984) and Crohn's disease (D'Agostino *et al*, 1988; Thompson *et al*, 1988).

*Correspondence: B Guida, Via Calata S. Francesco 12/b, 80127 Napoli, Italy.

Guarantor: B Guida.

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It has been suggested that the non-immunological forms of urticaria may be triggered by increased absorption or impaired metabolism of mediators such as histamine (Lesso, 1991). The aim of the present study was to evaluate histamine plasma levels as well as post-heparin DAO and intestinal permeability in patients with idiopathic chronic urticaria before and after an oligoallergenic diet.

Methods

We enrolled 10 patients (mean age 31.7 y, s.d. 12.7; three men and seven women) with chronic idiopathic urticaria not controlled by therapy (steroids, H1 anti-histamines and/or diet). Clinical manifestations of disease were pomphus, itch and angioedema. All patients, before starting the study, were on a free and uncontrolled diet. No patient complained of gastrointestinal symptoms. We evaluated post-heparin plasma DAO activity, jejunal mucosal permeability and histamine plasma levels before and after an oligoantigenic and histamine-free diet. Diagnosis of CIU was defined according to the criteria of Warin (1976).

Before entering the study, all patients were evaluated by prick skin tests for common food allergens and only negative patients were enrolled. Patients did not take any medication for 20 days before and during the study. Each patient was prescribed an individual restricted diet for 21 days and continued serial reintroduction for the following 70 days. Diet was normocaloric (according to RDA guidelines) and the proportion of carbohydrate, fat, and protein was 55–60%, 25–30%, and 15–20% respectively. Patients were evaluated before starting the diet, after 21 days of controlled diet and thereafter at days 60 and 90.

The control group for plasma levels histamine determinations included six healthy subjects. Informed consent was obtained from all patients and approval for the study was obtained from the university's standing committee on ethical practice.

Diet

Dietary procedures consisted of two different steps:

1. A 3 week period with a oligoantigenic (Metcalfe, 1984; Bahna, 1984) and histamine-free diet (Wantke, 1993). During this period the patients made a daily assessment of their symptoms by filling in a diary card and reporting symptoms. Symptoms (pomphus, itching, angioedema) were each scored daily using a numerical scale of severity (0, 1, 2, 3) in order of increasing severity.
2. A 10 week run-in period with a serial reintroduction of individual foods in normal amounts every other day. Foods were introduced according to antigenic characteristics of animals and vegetables (Sheldon *et al*, 1967).

During this period the patients continued to fill in the daily diary symptom score. Whenever a patient identified an offending food, this was eliminated from the diet and tested again at the end of the period, in order to make a definite diagnosis of intolerance. The well-tolerated foods were introduced in the patient's diet.

Plasma histamine assay

Blood was drawn in each patient, after an overnight fast, at about 8.30 a.m. Samples were immediately centrifuged at 4°C (1000 rpm for 10 min) and plasma was separated from blood cells. Proteins were precipitated by the addition of

10% perchloric acid and supernatant was stored at – 20°C. Histamine assay was performed in one run with an automated fluorometric technique (Siragarian, 1974; Patella *et al*, 1995a, b).

PHD test and DAO assay

Heparin (15 000 I.U.) was administered by an intravenous bolus. Blood (7 ml) was drawn 60 min after the injection, collected in heparinized tubes, and centrifuged at 3000 g. Plasma was stored at – 20°C and assayed for DAO within a week. Written informed consent was obtained from all patients. No complication resulted from the heparin bolus. Whenever the test was performed in outpatients, they were allowed to leave the hospital 4 h after the end of the test. DAO was assayed in triplicate by a ¹⁴C-putrescine method as described in a previous study. Briefly, the assay mixture contained: 1.5 ml sodium phosphate buffer, 0.1 M, pH 7.2; 0.3 ml sample; and 0.1 ml substrate, a mixture of putrescine dihydrochloride and 1–4 ¹⁴C-putrescine dihydrochloride up to a final concentration of 5 nmol per tube with an activity of 0.1 µCi. The samples were incubated for 60 min at 37°C. The labeled reaction product, ¹⁴C-1-pyridine, was directly extracted in a toluene-based scintillation mixture and assayed for ¹⁴C by a Packard Tricarb 4530 scintillation counter (Okuyama and Kobayashi, 1961). DAO activity was expressed as U/ml (1 U = 1 nmol of putrescine dihydrochloride oxidised in 1 h at 37°C pH 7.2). Based on previous studies, the normal range was considered to be 3.7–7.7 U/ml (D'Agostino *et al*, 1988).

Intestinal permeability

Intestinal permeability was evaluated by the cellobiose/mannitol sugar permeability test, as described by Strobel *et al* (1984). After an overnight fast, patients presented at about 8.30 a.m. Patients emptied the bladder and then drank a solution containing 5 g cellobiose, 2 g mannitol and 20 g sucrose, diluted in 200 ml sterile water. Urine was collected during the following 5 h. After recording the total urine volume, 10 ml aliquots were stored at – 20°C for cellobiose and mannitol assay. Mannitol was assayed by the methods of Corcoran and Page (1974). It was oxidized to formaldehyde by periodic acid. Formaldehyde reacts with chromotropic acid to form a purple complex which is measured at 570 nm absorbance. Cellobiose was assayed by the method described by Strobel *et al* (1984). Cellobiose reacts with β-glucosidase to yield two molecules of glucose. D-Glucose was measured using the hexokinase procedure with NADPH generation measured at 340 nm. For each of the two administered molecules cellobiose and mannitol, the percentage urinary recovery was calculated. The final ratio of percentage cellobiose recovery to percentage mannitol recovery (C/M ratio) was calculated. A C/M ratio lower than 37×10^{-3} was considered normal (Strobel *et al*, 1984).

Data were expressed as the mean ± s.d. Statistical analysis was performed by Student's *t*-test.

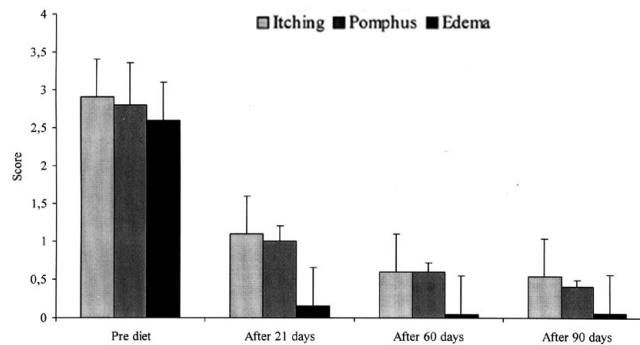
Results

The baseline patient characteristics are listed in Table 1. In all patients symptoms were greatly reduced after 3 weeks on the diet and continued to improve thereafter. Figure 1 shows the response to the dietary protocol.

Follow-up at 13 weeks showed complete remission in 3/10, partial remission in 3/10 and partial remission with transient relapses in 4/10. Patients in the latter group

Table 1 Baseline characteristics of subjects

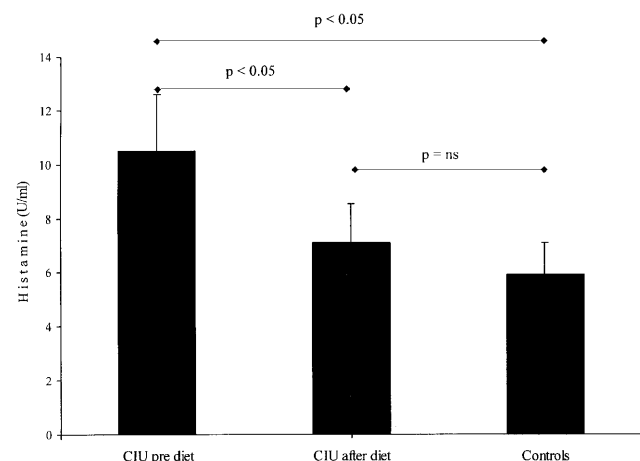
Characteristics	Data
All subjects (n)	10
Age (y \pm s.d.)	31.7 \pm 12.7
Male (n)	3
Female (n)	7
Duration of symptoms (months)	14.5 (7–60) ^a
Subjects with pomphus (n)	10
Subjects with itching (n)	10
Subjects with angioedema (n)	9

^aMedian; lower and upper values in parentheses.**Figure 1** Post treatment improvement of symptoms. Values recorded before starting the diet for itching, pomphus and edema (pre diet) were significantly higher ($P < 0.05$) than values observed after an oligoantigenic and histamine-free diet (after 21 days) and during serial controlled reintroduction of foods (after 60 days and after 90 days).

showed transient relapses that appeared to be correlated to emotion or intercurrent illnesses.

Histamine plasma levels in patients with CIU were significantly ($P < 0.05$) higher than controls and were significantly reduced after 21 days of the diet ($P < 0.05$; Figure 2).

Table 2 shows post-heparin DAO activity and intestinal permeability before and after 21 days of a controlled diet. Post-heparin DAO activity was slightly reduced or close to the lower limit of the normal range. Small intestinal permeability appeared normal in all patients. Neither DAO activity nor intestinal permeability showed any significant modification after diet.

**Figure 2** Histamine plasma levels in subjects with CIU before and after diet and in controls. Histamine plasma levels in patients with CIU were significantly ($P < 0.05$) higher than in controls and were significantly reduced after 21 days of diet ($P < 0.05$).**Table 2** DAO activity and intestinal permeability index at baseline and after the diet. Compared to normal values, post-heparin DAO activity was slightly reduced, while intestinal permeability was normal. Both parameters were not affected by the diet

	Value		
	Pre-diet ^a	Post-diet ^a	Normal values
DAO (U/ml) \pm s.d.	2.79 \pm 1.56	2.90 \pm 1.22	> 3.7
Permeability Index			
C/M(%) $\times 10^3 \pm$ s.d.	13.04 \pm 3.5	17.35 \pm 3.5	< 3.7

^aAfter 21 days of diet.

Discussion

In chronic idiopathic urticaria the possible pathogenic role of pseudoallergic reactions to food has been repeatedly discussed, but stringent prospective studies regarding their clinical significance are not available. An alternative pathogenic mechanism hypothesized (Lessof, 1991) is a reduction of the intestinal barrier against chemical compounds, such as histamine, contained in some foods that may trigger pseudoallergic reactions. In fact, many patients suffering from CIU attribute their symptoms to food intolerance (Julin, 1981; Sayag *et al*, 1988; Bischoff & Manns, 1998).

Increased levels of skin histamine have been reported in patients with CIU (Smith *et al*, 1995) while, to our knowledge, histamine plasma levels have not been previously evaluated.

In the present study we found higher histamine plasma levels in CIU patients on free diet compared to controls. After an oligoantigenic and histamine-free diet, plasma histamine levels fell to the same levels of the control group. This finding suggests that histamine plasma levels are dependent on diet in patients with chronic idiopathic urticaria.

Both an oligoantigenic and histamine-free diet (Wantke, 1993; Metcalfe, 1984) and an additive- and preservative-free diet (Zuberbier *et al*, 1995; Henz & Zuberbier, 1998) have been shown to induce significant clinical improvement in patients with CIU. In our study, the disappearance or reduction of symptoms after the diet appeared to be correlated with lower histamine plasma levels.

A deficient degradation of histamine by the enzyme diamine oxidase has been hypothesized as a possible cause of an increased absorption of histamine (Lessof, 1991). In our study, post-heparin DAO levels in CIU patients were slightly reduced compared to controls or in the lower part of the normal range. This observation may suggest a subclinical impairment of small bowel enterocyte function that can allow a higher absorption of histamine and higher histamine plasma levels. However, no significant modification of post-heparin DAO was found after an effective oligoantigenic and histamine-free diet, while a significant clinical improvement was recorded. A possible explanation to this discrepancy is that the diet improved symptoms only by reducing histamine load to the small bowel but did not alter the functional status of intestinal mucosa. However, the reason for relatively low DAO levels remains unexplained and will need further studies to be clarified.

Gastrointestinal permeability was normal in CIU patients and was not changed after the diet. This observation indicates that a morphological damage of intestinal mucosa should be excluded in CIU. In the present study,

the avoidance of the offending food(s) followed by a serial reintroduction of a 'low histamine' diet appeared to be effective to improve symptoms and reduce histamine plasma levels.

However, we can not establish whether clinical improvement was due to low levels of histamine or low levels of offending antigens in the diet. Furthermore, whether the low histamine plasma levels are caused by the reduced exogenous load or by a fall in mast cell histamine production remains unclear.

In conclusion, this study suggests that pseudo-allergic manifestations of CIU may be related to a subclinical functional impairment of the small bowel. This condition may result in a loss of the protective role of the intestinal mucosa against offending molecules in foods. While an increased histamine absorption caused by a reduction of small bowel DAO activity might be one of such mechanisms, we can not exclude a role for other chemical food compounds. Further studies are needed to better elucidate the pathogenic mechanisms of CIU and to evaluate the possible effects of foods on mast cell degranulation.

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